

What is claimed:

1. A method to produce glucosamine by fermentation, comprising:

(a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a microorganism having a genetic modification in an amino sugar metabolic pathway, said amino sugar metabolic pathway selected from the group consisting of a pathway for converting glucosamine-6-phosphate into another compound, a pathway for synthesizing glucosamine-6-phosphate, a pathway for transport of glucosamine or glucosamine-6-phosphate out of said microorganism, a pathway for transport of glucosamine into said microorganism, and a pathway which competes for substrates involved in the production of glucosamine-6-phosphate;

wherein said step of culturing produces a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said microorganism; and

(b) recovering said product.

2. The method of Claim 1, wherein said glucosamine-6-phosphate is intracellular and said glucosamine is extracellular, wherein said step of recovering comprises a recovering step selected from the group consisting of recovering said glucosamine-6-phosphate from said microorganism, recovering said glucosamine from said fermentation medium, and a combination thereof.

3. The method of Claim 1, wherein said product is glucosamine which is secreted into said fermentation medium by said microorganism and wherein said step of recovering comprises purification of said glucosamine from said fermentation medium.

4. The method of Claim 1, wherein said product is intracellular glucosamine-6-phosphate and said step of recovering comprises isolating said glucosamine-6-phosphate from said microorganism.

5. The method of Claim 1, wherein said product is intracellular glucosamine-6-phosphate and said step of recovering further comprises dephosphorylating said glucosamine-6-phosphate to produce glucosamine.

6. The method of Claim 1, wherein said step of culturing comprises maintaining said source of carbon at a concentration of from about 0.5% to about 5% in said fermentation medium.

7. The method of Claim 1, wherein said step of culturing is performed at a temperature from about 28°C to about 37°C.

8. The method of Claim 1, wherein said step of culturing is performed in a fermentor.

9. The method of Claim 1, wherein at least about 1 g/L of said product is recovered.

10. The method of Claim 1, wherein said genetic modification is a modification in a nucleic acid molecule encoding a protein selected from the group consisting of *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme II^{Nag}, glucosamine-6-phosphate synthase, phosphoglucosamine mutase, glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase, Enzyme II^{Glc} of the PEP:glucose PTS, EIIM,P/III^{Man} of the PEP:mannose PTS, and a phosphatase.

11. The method of Claim 1, wherein said microorganism has a genetic modification that increases glucosamine-6-phosphate synthase action.

12. The method of Claim 11, wherein said genetic modification results in overexpression of glucosamine-6-phosphate synthase by said microorganism.

13. The method of Claim 11, wherein said genetic modification comprises transformation of said microorganism with a recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase which has glucosamine-6-phosphate synthase enzymatic activity, wherein said recombinant nucleic acid molecule is operatively linked to a transcription control sequence.

14. The method of Claim 13, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence encoding a homologue of glucosamine-6-phosphate synthase.

15. The method of Claim 13, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence encoding amino acid sequence SEQ ID NO:16.

16. The method of Claim 13, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15.

17. The method of Claim 13, wherein said recombinant nucleic acid molecule comprises a nucleic acid molecule selected from the group consisting of pKLN23-28, nglmS-28₂₁₃₄ and nglmS-28₁₈₃₀.

18. The method of Claim 13, wherein said recombinant nucleic acid molecule is integrated into the genome of said microorganism.

19. The method of Claim 13, wherein said recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase comprises a genetic modification which increases the action of said glucosamine-6-phosphate synthase.

20. The method of Claim 19, wherein said recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase comprises a genetic modification which reduces glucosamine-6-phosphate product inhibition of said glucosamine-6-phosphate synthase.

21. The method of Claim 19, wherein said genetic modification results in at least one amino acid modification selected from the group consisting of deletion, insertion, inversion, substitution and derivatization of at least one amino acid residue of said glucosamine-6-phosphate synthase, said at least one amino acid modification resulting in increased glucosamine-6-phosphate synthase action.

22. The method of Claim 21, wherein said at least one amino acid modification is at an amino acid sequence position, corresponding to amino acid sequence SEQ ID NO:16, selected from the group consisting of Ile(4), Ile(272), Ser(450), Ala(39), Arg(250), Gly(472), Leu(469), and combinations thereof.

23. The method of Claim 22, wherein said amino acid modification is a substitution selected from the group consisting of:

(a) an amino acid residue having an aliphatic hydroxyl side group for Ile(4);

(b) an amino acid residue having an aliphatic hydroxyl side group for Ile(272);

(c) an amino acid residue having an aliphatic side group for Ser(450);

10 (d) an amino acid residue having an aliphatic hydroxyl side group for Ala(39);

(e) an amino acid residue having a sulfur-containing side group for Arg(250);

15 (f) an amino acid residue having an aliphatic hydroxyl side group for Gly(472);

(g) an amino acid residue having an aliphatic side group for Leu(469);

(h) and combinations of (a)-(g).

24. The method of Claim 22, wherein said amino acid modification is a substitution selected from the group consisting of: Ile(4) to Thr, Ile(272) to Thr, Ser(450) to Pro, Ala(39) to Thr, Arg(250) to Cys, Gly(472) to Ser, 5 Leu(469) to Pro, and combinations thereof.

25. The method of Claim 22, wherein said amino acid modification is a substitution of a proline residue for a leucine residue at amino acid sequence position Leu(469).

26. The method of Claim 22, wherein said amino acid modification is a substitution of an amino acid residue selected from the group consisting of:

5 (a) a threonine residue for an alanine residue at position Ala(39);

(b) a cysteine residue for an arginine residue at position Arg(250);

(c) a serine residue for a glycine residue at position Gly(472); and

10 (d) any combination of (a), (b), or (c).

27. The method of Claim 22, wherein said amino acid modification is a substitution selected from the group consisting of:

(a) a threonine residue for an isoleucine residue
5 at position Ile(4);

(b) a threonine residue for an isoleucine residue
at position Ile(272);

(c) a proline residue for a serine residue at
position Ser(450); and

10 (d) any combination of (a), (b), or (c).

28. The method of Claim 19, wherein said recombinant
nucleic acid molecule comprises a nucleic acid sequence
encoding a glucosamine-6-phosphate synthase comprising an
amino acid sequence selected from the group consisting of SEQ
5 ID NO:19, SEQ ID NO:22, SEQ ID NO:25, SEQ ID NO:28 and SEQ ID
NO:31.

29. The method of Claim 19, wherein said recombinant
nucleic acid molecule comprises a nucleic acid sequence
selected from the group consisting of SEQ ID NO:17, SEQ ID
NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24,
5 SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:29 and SEQ ID NO:30.

30. The method of Claim 19, wherein said recombinant
nucleic acid molecule comprises a nucleic acid molecule
selected from the group consisting of pKLN23-49, pKLN23-54,
pKLN23-124, pKLN23-149, pKLN23-151, nglms-49₂₁₈₄, nglms-49₁₈₃₀,
5 nglms-54₂₁₈₄, nglms-54₁₈₃₀, nglms-124₂₁₈₄, nglms-124₁₈₃₀, nglms-
149₂₁₈₄, nglms-149₁₈₃₀, nglms-151₂₁₈₄ and nglms-151₁₈₃₀.

31. The method of Claim 19, wherein said recombinant
nucleic acid molecule is integrated into the genome of said
microorganism.

32. The method of Claim 11, wherein said microorganism
has at least one additional genetic modification in a gene
encoding a protein selected from the group consisting of *N*-
acetylglucosamine-6-phosphate deacetylase, glucosamine-6-

phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme II^{Nag}, phosphoglucosamine mutase, glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase, Enzyme II^{Glc} of the PEP:glucose PTS, and EIIM,P/III^{Man} of the PEP:mannose PTS, wherein said genetic modification decreases the action of said protein.

33. The method of Claim 11, wherein said microorganism has at least one additional genetic modification in a gene encoding a phosphatase, wherein said genetic modification increases the action of said phosphatase.

34. The method of Claim 11, wherein said microorganism has additional modifications in genes encoding the following proteins: *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase and *N*-acetyl-glucosamine-specific enzyme II^{Nag};

wherein said genetic modification decreases the action of said proteins.

35. The method of Claim 34, wherein said genetic modification is a deletion of at least a portion of said genes.

36. The method of Claim 1, wherein said microorganism is selected from the group consisting of bacteria and yeast.

37. The method of Claim 1, wherein said microorganism is a bacterium of the genus *Escherichia*.

38. The method of Claim 1, wherein said microorganism is *Escherichia coli*.

39. The method of Claim 38, wherein said genetic modification is a mutation in an *Escherichia coli* gene selected from the group consisting of *nagA*, *nagB*, *nagC*, *nagD*,

nagE, *manXYZ*, *glmM*, *pfkB*, *pfkA*, *glmU*, *glmS*, *ptsG* and a
phosphatase gene.

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40. A method to produce glucosamine by fermentation, comprising:

(a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, an *Escherichia coli* transformed with a recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase, wherein said recombinant nucleic acid molecule increases glucosamine-6-phosphate synthase action in said *Escherichia coli*, and wherein said recombinant nucleic acid molecule is operatively linked to a transcription control sequence;

wherein said step of culturing produces a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said *Escherichia coli*; and

(b) recovering said product.

41. The method of Claim 40, wherein said *Escherichia coli* has at least one additional genetic modification in at least one gene selected from the group consisting of *nagA*, *nagB*, *nagC*, *nagD*, *nagE*, *manXYZ*, *glmM*, *pfkB*, *pfkA*, *glmU*, *glmS*, *ptsG* and a phosphatase gene.

42. The method of Claim 40, wherein said at least one additional genetic modification comprises a deletion of *nagA*, *nagB*, *nagC*, *nagD*, *nagE*, and a mutation in *manXYZ*, said modification resulting in decreased action of *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase and *N*-acetyl-glucosamine-specific enzyme II^{Nag}.

43. A microorganism for producing glucosamine by a biosynthetic process, said microorganism being transformed with a recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase, said recombinant nucleic acid molecule being operatively linked to a transcription control sequence and comprising a genetic modification which increases the action of said glucosamine-6-phosphate synthase;

wherein expression of said recombinant nucleic acid molecule increases production of glucosamine by said microorganism.

44. The microorganism of Claim 43, wherein said microorganism has at least one additional genetic modification in a gene encoding a protein selected from the group consisting of *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme II^{Nag}, phosphoglucosamine mutase, glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase. Enzyme II^{Glc} of the PEP:glucose PTS, and EIIM,P/III^{Man} of the PEP:mannose PTS, wherein said genetic modification decreases the action of said protein.

45. The microorganism of Claim 43, wherein said microorganism has at least one additional genetic modification in a gene encoding a phosphatase, wherein said genetic modification increases the action of said phosphatase.

46. The microorganism of Claim 43, wherein said microorganism is *Escherichia coli* which has at least one additional genetic modification in a gene selected from the group consisting of *nagA*, *nagB*, *nagC*, *nagD*, *nagE*, *manXYZ*, *glmM*, *pfkB*, *pfkA*, *glmU*, and *ptsG*, wherein said genetic

modification decreases the action of a protein encoded by said gene.

47. The microorganism of Claim 43, wherein said microorganism is *Escherichia coli* which has a deletion of *nag* regulon genes.

48. The microorganism of Claim 43, wherein said microorganism is *Escherichia coli* which has a deletion of *nag* regulon genes and a genetic modification in *manXYZ* genes such that proteins encoded by said *manXYZ* genes have decreased action.

49. The microorganism of Claim 43, wherein said microorganism produces at least about 1 g/L of glucosamine when cultured from about 10 to about 60 hours at from about 28°C to about 37°C to a cell density of at least about 8 g/L by dry cell weight, in a pH 7.0 fermentation medium comprising: 14 g/L K_2HPO_4 , 16 g/L KH_2PO_4 , 1 g/L $Na_3Citrate \cdot 2H_2O$, 5 g/L $(NH_4)_2SO_4$, 20 g/L glucose, 10 mM $MgSO_4$, 1 mM $CaCl_2$, and from about 0.2mM to about 1 mM IPTG.

50. A microorganism for producing glucosamine by a biosynthetic process, said microorganism comprising:

(a) a recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase, said recombinant nucleic acid molecule being operatively linked to a transcription control sequence, wherein expression of said recombinant nucleic acid molecule increases the action of glucosamine-6-phosphate synthase by said microorganism; and,

(b) at least one genetic modification in a gene encoding a protein selected from the group consisting of *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme II^{Na_g}, phosphoglucosamine mutase, glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase, Enzyme II^{Glc} of the PEP:glucose PTS, and EIIM,P/III^{Man} of the PEP:mannose PTS, wherein said genetic modification decreases the action of said protein.

51. The microorganism of Claim 50, wherein said microorganism has at least one additional genetic modification in a gene encoding a phosphatase, wherein said genetic modification increases the action of said phosphatase.

52. A recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a glucosamine-6-phosphate synthase having a genetic modification that results in increased glucosamine-6-phosphate synthase action.

53. The recombinant nucleic acid molecule of Claim 52, wherein said genetic modification results in at least one amino acid modification selected from the group consisting of deletion, insertion, inversion, substitution and derivatization of at least one amino acid residue of said glucosamine-6-phosphate synthase, said at least one amino acid modification resulting in increased glucosamine-6-phosphate synthase action.

54. The recombinant nucleic acid molecule of Claim 52, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence encoding a glucosamine-6-phosphate synthase comprising an amino acid sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:25, SEQ ID NO:28 and SEQ ID NO:31.

55. The recombinant nucleic acid molecule of Claim 52, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:29 and SEQ ID NO:30.

56. A glucosamine-6-phosphate synthase which has glucosamine-6-phosphate synthase action, said synthase being encoded by a nucleic acid sequence having a genetic
5 modification that results in increased glucosamine-6-phosphate synthase action.

57. The glucosamine-6-phosphate synthase of Claim 56, wherein said synthase comprises at least one amino acid modification selected from the group consisting of deletion, insertion, inversion, substitution and derivatization of at least one amino acid residue.

58. The glucosamine-6-phosphate synthase of Claim 56, wherein said synthase is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:29 and SEQ ID NO:30.

59. The glucosamine-6-phosphate synthase of Claim 56, wherein said synthase comprises an amino acid sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:25, SEQ ID NO:28 and SEQ ID NO:31.

60. A method to produce glucosamine by fermentation, comprising:

(a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a genetically modified microorganism having increased glucosamine-6-phosphate synthase action, wherein said genetically modified microorganism is produced by a process comprising the steps of:

(1) generating modifications in an isolated nucleic acid molecule comprising a nucleic acid sequence encoding glucosamine-6-phosphate synthase to create a plurality of modified nucleic acid sequences;

(2) transforming microorganisms with said modified nucleic acid sequences to produce genetically modified microorganisms;

(3) screening said genetically modified microorganisms for glucosamine-6-phosphate synthase action; and,

(4) selecting said genetically modified microorganisms which have increased glucosamine-6-phosphate synthase action;

wherein said step of culturing produces a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said microorganism; and,

(b) recovering said product.

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